# Echinococcosis, toxocarosis and toxoplasmosis screening in a rural community in eastern Kazakhstan

Torgerson, P R ; Rosenheim, K ; Tanner, I ; Ziadinov, I ; Grimm, F ; Brunner, M ; Shaiken, S ; Shaikenov, B ; Rysmukhambetova, A ; Deplazes, P

Abstract: Objective To determine the extent of carnivore-transmitted parasitic zoonoses in a community in eastern Kazakhstan, a region where cystic echinococcosis (CE) re-emerged in recent years. Methods Cross sectional ultrasound study of 3126 human subjects to determine the extent of human cystic and alveolar echinococcosis (AE). Blood samples were taken from each subject and analysed for antibodies against Echinococcus, Toxocara and Toxoplasma spp. Each subject was questioned about possible risk factors that might be associated with zoonotic transmission. Analysis employed a mixed modelling approach based on the results of the ultrasound study, the serological results and the results of the questionnaire. Bayesian techniques were employed to estimate diagnostic performance. A helminthological study of the local dog population was also undertaken. Results A total of 23 subjects tested positive for CE on ultrasound and a further three individuals had strong serological evidence of infection. Another 24 reported treatment for CE. Ultrasound lesions or treatment for CE were associated with poverty. No ultrasound evidence of AE was found, but one individual had strong serological evidence of exposure to Echinococcus multilocularis. Toxoplasma seropositivity (16%; 504 individuals) increased with age. Household level Toxoplasma-seropositivity was associated with unsafe drinking water. Toxocara seropositivity (11%; 349 individuals) was more frequent in children and in individuals who disposed of dog faeces on the vegetable garden. A purgation study of dogs indicated that 13% of dogs in the community were infected with Echinococcus granulosus, 5% with E. multilocularis and 2% with Toxocara canis respectively. Conclusions There is significant transmission of E. granulosus to humans in this community. Transmission may be associated with poverty. There is little evidence of E. multilocularis transmission to humans, despite the presence in the parasite in the domestic dog population. Toxoplasma is actively transmitted and there is evidence for transmission by the water supply. Children are at highest risk of exposure to Toxocara

DOI: https://doi.org/10.1111/j.1365-3156.2009.02229.x

Editorial Manager(tm) for Tropical Medicine & International Health
Manuscript Draft

Manuscript Number: TMIH-D-08-00455R1

Title: Echinococcosis, toxocarosis and toxoplasmosis screening in a rural community in eastern Kazakhstan

Article Type: Original Research Paper

Keywords: Echinococcus, Kazakhstan, epidemiology, Toxoplasma, Toxocara

Corresponding Author: Dr Paul Torgerson,

Corresponding Author's Institution:

First Author: Paul Torgerson

Order of Authors: Paul Torgerson; Kathy Rosenheim; Isabelle Tanner; Iskender Ziadinov; Felix Grimm; Matthias Brunner; Sholpan Shaiken; Blok Shaikenov; Aizhan Rysmukhambetova; Peter Deplazes

Abstract: OBJECTIVES To determine the extent of carnivore-transmitted parasitic zoonoses in a community in eastern Kazakhstan: a region where there has been a re-emergence of cystic echinococcosis in recent years.
METHODS A cross sectional ultrasound study was undertaken on 3126 human subjects to determine the extent of human cystic and alveolar echinococcosis. Blood samples were taken from each subject and analysed for antibodies against Echinococcus, Toxocara and Toxoplasma spp. A survey was administered to each subject with questions about possible risk factors that might be associated with zoonotic transmission. A mixed modelling approach was undertaken based on the results of the ultrasound study, the serological results and the results of the questionnaire. Bayesian techniques were employed to estimate diagnostic performance. A helminthological study of the local dog population was also undertaken.

RESULTS A total of 23 subjects were CE-positive on ultrasound (US) and a further 3 had strong serological evidence of infection. Additionally 24 reported treatment for CE. US lesions or treatment for CE were associated with poverty. No US-evidence of alveolar echinococcosis was found but one individual had strong serological evidence of exposure to E. multilocularis. Toxoplasma-seropositives (504 individuals-16%) increased with age. Household level Toxoplasma-seropositivity was associated with unsafe drinking water. Toxocara-seropositivity (349 individuals-11%) was higher in children and in individuals who disposed of dog faeces on the vegetable garden. A purgation study of dogs indicated 13%, 5% and 2% of dogs in the community were infected with E. granulosus E. multilocularis and adult Toxocara canis, respectively.
CONCLUSIONS There is significant transmission of E. granulosus to humans in this community Transmission may be associated with poverty. There is little evidence of E. multilocularis transmission to humans, despite the presence in the parasite in the domestic dog population. Toxoplasma is actively transmitted and there is evidence for transmission by the water supply. Children are at highest risk of exposure to Toxocara.

# Echinococcosis, toxocarosis and toxoplasmosis screening in a rural community in eastern Kazakhstan

Paul R. Torgerson[1,3 *], Kathy Rosenheim[1], Isabelle Tanner[1], Iskender Ziadinov[1], Felix Grimm[1], Matthias Brunner[1], Sholpan Shaiken[2], Blok Shaikenov[2], Aizhan Rysmukhambetova[2], Peter Deplazes[1]

1. Institute of Parasitology, *WHO Collaborating Centre for Parasitic Zoonoses,* University of Zurich, Winterthurestrasse 266a, Zurich, Switzerland
2. Institute of Zoology, Academgorodok, Al Farabia Street, Almaty, Kazakhstan
3. Ross University School of Veterinary Medicine, PO Box 334, Basseterre, St Kitts, West Indies

*Corresponding author. email: ptorgerson@rossvet.edu.kn

Fax: +1 869 465 1203

Tel: +1 869 465 4161 Extension 395

.

**Key words**: *Echinococcus granulosus*, *Echinococcus multilocularis*, epidemiology, diagnosis, *Toxocara*, Toxoplasmosis

**Summary**

OBJECTIVES To determine the extent of carnivore-transmitted parasitic zoonoses in a community in eastern Kazakhstan: a region where there has been a re-emergence of cystic echinococcosis in recent years.

METHODS A cross sectional ultrasound study was undertaken on 3126 human subjects to determine the extent of human cystic and alveolar echinococcosis. Blood samples were taken from each subject and analysed for antibodies against *Echinococcus*, *Toxocara* and T*oxoplasma* spp. A survey was administered to each subject with questions about possible risk factors that might be associated with zoonotic transmission. A mixed modelling approach was undertaken based on the results of the ultrasound study, the serological results and the results of the questionnaire. Bayesian techniques were employed to estimate diagnostic performance. A helminthological study of the local dog population was also undertaken.

RESULTS A total of 23 subjects were CE-positive on ultrasound (US) and a further 3 had strong serological evidence of infection. Additionally 24 reported treatment for CE. US lesions or treatment for CE were associated with poverty. No US-evidence of alveolar echinococcosis was found but one individual had strong serological evidence of exposure to *E. multilocularis*. *Toxoplasma*–seropositives (504 individuals-16%) increased with age. Household level *Toxoplasma*-seropositivity was associated with unsafe drinking water. *Toxocara*-seropositivity (349 individuals-11%) was higher in children and in individuals who disposed of dog faeces on the vegetable garden. A purgation study of dogs indicated 13%, 5% and 2% of dogs in the community were infected with *E. granulosus E. multilocularis* and adult *Toxocara canis,* respectively.

CONCLUSIONS There is significant transmission of *E. granulosus* to humans in this community Transmission may be associated with poverty. There is little evidence of *E. multilocularis* transmission to humans, despite the presence in the parasite in the domestic dog population. *Toxoplasma* is actively transmitted and there is evidence for transmission by the water supply. Children are at highest risk of exposure to *Toxocara*.

K**eywords** *Echinococcus*, Kazakhstan, epidemiology, T*oxoplasma*, *Toxocara*.

## Introduction

Human cystic echinococcosis (CE) causes a high global burden of disease (Budke *et al.* 2006) and is re-emerging in Kazakhstan following the collapse of the Soviet Union (Torgerson *et al.* 2002). Human incidence, based on reports of surgical cases referred to regional centres, may substantially underestimate the true numbers of clinical cases. In Uzbekistan the true surgical incidence is four fold higher than official figures (Nazirov *et al.* 2002). There is little data concerning the prevalence of CE in rural Kazakhstan, although a report of a cross sectional ultrasound study from Kyrgyzstan suggested an ultrasound prevalence rate of 1.35% and prevalences of *Echinococcus granulosus* of 23% and 6% in farm and village dogs respectively have been reported in southern Kazakhstan (Torgerson *et al.* 2003a,b).

*Echinococcus multilocularis* is endemic to Kazakhstan (Shaikenov 2006) but human alveolar echinococcosis (AE) is rarely reported. Recently, 6 of 131 dogs from south-east Kazakhstan were shown to be infected with *E. multilocularis* (Stefanic *et al.* 2004) and high dog prevalences have been recorded from neighbouring Kyrgyzstan (Ziadinov *et al.* 2008). In China, contact with dogs is a known risk factor for AE (Craig 2006).

Little is known of the epidemiology of toxoplasmosis and toxocarosis in Kazakhstan. Toxocarosis is normally assumed to infect humans by consumption of embryonated eggs although transmission through undercooked meat is suggested (Hoffmeister, *et al.* 2007). *Toxocara canis* is believed to be the main cause of human toxocarosis, but *T. cati* may be important (Fisher 2003). Transmission of *Toxoplasma gondii* to humans occurs via environmental oocyst contamination or meat (Hill *et al.* 2005, Tenter *et al.* 2000). At least three outbreaks of toxoplasmosis have been attributed to waterborne transmission of oocysts (Karanis *et al.* 2007).

To gain insights into the epidemiology of carnivore transmitted parasitic zoonoses in rural Kazakhstan an ultrasound and serological survey of 3126 subjects in a community was initiated. Furthermore, a helminthological survey of dogs in this community was undertaken.

## Methods

### Study design and data collection

The cross sectional study, having received prior approval from the local health authorities, was undertaken in Jalanash in July-August 2005. Jalanash is located at grid reference $43^{o}$ north, $78^{o}$ east with a population of approximately 4,000.

The study area was divided into districts and all inhabitants were invited to participate by visiting households. A survey of the entire population was attempted. The observed gender and age distribution was compared to the expected distribution estimated from census data. Subjects were administered a questionnaire by interview in Russian or Kazakh. The questionnaire included risk factors for the transmission of echinococcosis and other zoonoses with details of dog, cat and livestock ownership and possible contact with foxes through hunting and skinning. Dog owners were asked the length of ownership, if they fed offal to their dogs, if home slaughtering was practiced and if their dogs received anthelmintics. The subjects were also asked if they smoked, the number of days they were sick in the last year, their living standard and family history of echinococcosis. Informed consent was obtained before the subject was examined. Children were only examined with the parental consent. A full ultrasound scan of the abdominal region was undertaken. Any CE lesions that were detected by ultrasound were classified as CE1-CE5 (WHO 2002). Any subjects with evidence of echinococcosis were arranged a referral for further treatment. Finger prick blood (200 ul) was collected from subjects with a system for capillary blood collection (Microvette R Potasium-EDTA, Sarsted AG, Sevelen/SG, Switzerland). Samples were stored at -20 °C until further analysis.

### Laboratory Analysis

Samples were analyzed at a 1:100 dilution with standard ELISA procedures using cyst fluid antigens of *E. granulosus* (EgHF), the Em2G11-antigen of *E. multilocularis* (Deplazes & Gottstein 1991), excretory/secretorary antigens of *Toxocara canis* (Gottstein & Speiser 1984) and a commercially available *Toxoplasma gondii* tachyzoite antigen preparation (Institute Virion Ltd., Rüschlikon, Switzerland). Rabbit anti-human IgG antibodies specific for the $C_H2$ domain of the gamma chain (DAKO, A0089) coupled to

alkaline phosphatase (Roche Diagnostics, 567 752,) were used as detection antibodies. Optimal antigen concentrations and detection antibodies were determined by checkerboard titrations (data not shown).Except for anti-*Toxoplasma* antibodies, threshold values for tests were defined as the mean OD plus 3 standard deviations from analysis of 50 healthy Swiss blood donors. For the determination of anti-*Toxoplasma* antibodies, a panel of specified sera that contained 0, 6 (threshold), 100, and 200 International Units of anti-*Toxoplasma* antibodies as determined by a commercially available test kit (Platelia® Toxo IgG TMB Testkit, Bio Rad Laboratories Inc.) was included in each run. In addition, 25 negative and 25 positive samples obtained in the present study were tested with the same results in this test kit. All samples with a positive result in the EgHF-ELISA were further tested on Western blots (AgB-WB) for the presence of antibodies to *E. granulosus* antigen-B (AgB) (Oriol *et al.* 1971, Ortona *et al.* 2000). Individuals who were positive for the ELISA and positive on AgB-WB were advised to have a follow up examination for echinococcosis at a referral centre.

**Study of the dog population**

Dogs were treated with arecoline hydrobromide as described previously (Torgerson *et al.* 2003). Total counts of the parasites recovered from purge material were recorded.

**Statistical analysis**

Human data was analysed in R ( R 2.4.0. The R Foundation for Statistical Computing, http://CRAN.R-project.org). A mixed modeling technique was adopted with random effects at the level of village district and fixed effects at subject level. Firstly the presence of ultrasound lesions and/or recent history of treatment was analysed as the dependent variable with other variables. Seropositivity to *Toxocara* or *Toxoplasma* was also examined as dependent variables.

At the household level households with at least one case of CE diagnosed by ultrasound and/or history of treatment was considered positive while households only having non affected individuals was considered negative. Households were also similarly classified according to seropositivity to *Toxoplasma* or *Toxocara*. The total dog or cat population was estimated from animals reported at the household level. A backward

stepwise logistic regression analysis with factors having a significance of >0.2 being removed form the model. The final model was adopted with all remaining factors with a $p<0.05$ indicating significant factors.

A Bayesian approach was used to estimates the diagnostic performance. Subjects positive for the EgHF-ELISA were further tested by AgB Western blot (AgB-WB) (Ortona *et al.* 2000). For this analysis patients reporting a history of treatment for echinococcosis were excluded from the analysis (n= 24). AgB-WB has a sensitivity and specificity of 66% and 100%, respectively (Ortona *et al.* 2000). Therefore, EgHF-ELISA followed by AgB-WB confirmation had a specificity of 100% for echinococcosis. As there is knowledge about 2 parameters (of the 2 parameters of sensitivity, 2 of specificity and prevalence) than estimates of the other three parameters can be made (Dendukuri & Joseph 2001).Cross reactivity between AE and CE is possible as 40% of AE patients are AgB-WB positive (Mamuti, et al. 2005). Two scenarios were then analysed. The first assumed that US had a specificity of 100%. The second proposed US specificity was 95.6% (del Caprio *et al.* 2000). Marcov Chain Monte Carlo with Gibbs sampling was undertaken in Excel (Rapsch *et al.* 2006). Ultrasound diagnosis and serology were assumed to be conditionally independent. For unknown parameters non-informative priors were used. For both scenarios the diagnostic parameters of the EgHF-ELISA were also estimated if it had been the only test available, but using the results of the other test procedures to give information about the infection status of each individual in the population.

## Results

### Data on the population

A total of 3126 subjects were examined. The average household size was 5.5 persons. There was evidence of bias concerning gender and age (table 1).

In total 2984 subjects reported close contact with dogs (owning dogs now or in the preceding five years or caring for neighbours' dogs). Only 9 subjects allowed their dogs in the house. The dog population was estimated at 973 with a mean of 1.7 dogs per household. Likewise the cat population was estimated at 178.

Twenty three cases of CE were detected by ultrasound survey (0.7%). Of these 15 had active CE lesions (CE1-CE3) and 8 had evidence of CE4 or CE5 lesions. Eleven of these cases had not previously heard of CE. An additional 24 cases reported treatment for CE giving a total of 47 cases with CE or a history of CE (1.5%). No cases of AE were detected.

Seventy four subjects were seropositive in the EgHF-ELISA. Of these 22 either had a history of treatment for CE or had CE lesions detectable by ultrasound. The remaining 52 subjects had no clinical evidence or history of CE (table 2). Of these 3 were confirmed as probable exposed by being positive to AgB-WB. Four subjects were positive to the Em2G11 antigen of which one was also positive to EgHF-antigen. All four cases were negative on AgB-WB. The subject which was seropositive to EgHF- and Em2G11-antigen was likely exposed to *E. multilocularis*. The 3 cases which were positive to Em2G11 but negative to hydatid fluid cyst antigen and AgB were classified as AE negative. Of the active lesions (CE1-CE3) 10 were positive for the EgHF-ELISA (66%). Of the 7 inactive lesions (CE4 or CE5) 1 was positive for the EgHF-ELISA (14.3%) ($p=0.03$, Fishers Exact Test compared to active lesions). Of the 24 cases with a history of CE, 11 were positive by EgHF-ELISA (46%) (n.s. compared to active lesions). Of 3078 patients that had no history for CE and were ultrasound negative, 52 were seropositive by EgHF-antigen. Of these three were also positive by AgB-WBs.

Twenty subjects had been treated for hepatic CE and 4 for pulmonary CE. Of these 12 had been recorded in the previous 5 years. Assuming all previously treated cases attended for US examination, the annual incidence of new cases is approximately 50 cases per 100,000 between 2000 and 2005 based on the size of the target population. The numbers of seropositive cases treated between 2000 and 2005 was 6 compared to 5 in cases treated earlier. Three of the 4 pulmonary cases had been treated since 2000. There were 2 seropositive pulmonary cases, treated in 2003 and 2005.

The results of the ultrasound and serology are given in table 2 with the estimated diagnostic parameters of the test procedures and the population prevalence with 95% credible intervals given in table 3 and 4. As there was very little evidence of AE transmission the results refer to diagnosis of CE

In total 505 subjects (16.1%) were seropositive for *Toxoplasma* and 349 showed specific antibodies in the *Toxocara E/S antigen-ELISA* (11.1%).

**Logistic regression**

Only a poor living standard was significantly associated with an ultrasound diagnosis of CE lesion (n=23) (regression coefficient 0.98, odds ratio 2.66, CI 1.24-5.71, p=0.01). When this group was combined with those reporting a history of CE treatment (n=47) there were significant associations with poor living standard, household size and the annual numbers of days sick (table 5).

There was no evidence of clustering CE within households and no difference in household size between good and poor living standards (data not shown). At the household level, the only significant variable was household size (table 6).

Seropositivity to *Toxocara* was significantly associated with being male, poor offal disposal practices and putting dog faeces on the garden and inversely associated with smoking and age. The mixed effect model had a lower AIC indicating some clustering of seropositivity at the level of the town district (table 7).Household level analysis suggested a significant association a higher household living standard (table 6). There was no evidence of clustering within households.

Seropositivity to *Toxoplasma* increased with age (table 8). There was also an association with seropositivity to CE. The random effects model gave a lower AIC indicating clustering of infection at the district level. There was no evidence of clustering within households Seropositivity of at least one member of the household to toxoplasmosis was associated with household water supply from a stream, with smaller numbers of cattle, household *Toxocara* seropositivity and household size (table 6).

**Canine helminthological study**

A total of 632 dogs were studied between 2003 and 2005. The mean abundance of *E. granulosus* was 812 parasites per dog and 13% of animals were infected. Fewer dogs were found to be infected with *E. multilocularis* (5%) and the abundance was 72 parasites per dog. The results of the helminth fauna are illustrated in table 9.

**Discussion**

This study describes the prevalence and seroprevalence of important carnivore transmitted parasitic zoonoses in a rural population in eastern Kazakhstan. Bias in the population data may be due to men being unavailable for examination during the working day. This community also has high levels of poverty and some men are absent due to working in larger cities such as Almaty. Furthermore not every member of households represented in the study presented for the survey.

Forty seven cases (1.5%) had either CE lesions detectable by ultrasound (n=23) or a history of CE (n=24). AE was not detected by ultrasound although there were 4 positive cases in the Em2G11-ELISA including one which was positive to both hydatid cyst fluid antigen and Em2G11-antigen. The sensitivity of the EgHF antigen ELISA for detecting AE has been reported as 97.1%, and experimental *E. multilocularis* infections in pigs can be detected one month after egg inoculation (Deplazes et *al.* 2005). The specificity of Em2-antigen approaches 100% (Gottstein *et al.* 1993). However a cut-off of the mean plus three standard deviations of a sample of healthy Swiss blood donors was used. This suggests a specificity of 99.9%. Therefore in a population of 3126, three false positives would be expected. The low OD values of 3 patients to the EgHF-antigen indicate that they are likely false positives.

The prevalence of undiagnosed CE within the population was either 1.1% or 0.5% depending on the model assumptions. If recently treated cases of CE are then added back, there is an estimated prevalence of either 1.9% or 1.3%. Few if any non-CE lesions were mistakenly given the diagnosis of CE as there were two ultrasound operators, each lesion was photographed and rechecked and classified as CE1-CE5. All cases that were diagnosed as CE were referred for further investigation but the results of these are unavailable to confirm our findings. The lower limit for the specificity of the ultrasound is 99.3% if all 23 ultrasound positive individuals were false positives: higher than the prior used in the analysis. AgB-WB confirms the diagnosis of 8 of the ultrasound positive cases, resulting in a lower limit to the specificity of 99.5%. The AgB-WB is known to be genus rather than species specific for the diagnosis of echinococcosis (Mamuti et *al.*

2005). This must be considered when there is co-endemicity of AE and CE. However, there was negligible evidence of AE in this study and no subjects were positive to both the AgB-WB and the Em2G11-ELISA. The study of del Caprio *et al.* (2000) indicated 3 ultrasound false positives in 66 individuals without CE. Using this data as the prior the median estimate of the specificity was shifted to the minimum possible under the model assumptions.

The poor positive predictive value and sensitivity of EgHF-ELISA for diagnosis of CE illustrates the dangers of using serology as a mass screening test without ultrasound. However, if combined with ultrasound and EgHF-ELISA positives are confirmed using AgB-WB, the combined sensitivity of 83% or 93% for the two scenarios is suggested. Missing cases include small cysts, or cysts in non-abdominal locations in individuals not seroconverting. In this study 2 of 4 individuals reporting previous treatment for pulmonary CE had seroconverted. The apparent low sensitivity of ultrasound of 67 or 69% could be because patients with more advanced lesions had been treated. This would lower the sensitivity of ultrasound in comparison to a site, such as the Tibetan plateau, where few treatment options are available. The analysis also assumed that the two diagnostic procedures were conditionally independent (Dendukuri & Joseph 2001). However, with the available data this assumption cannot be tested.

The EgHF-ELISA detected a greater proportion of patients with active lesions (CE1-CE3) than inactive lesions (CE4-CE5). The seropositive rate was not significantly higher in subjects with active lesions compared to those with a history of treatment. Indeed 5 of 12 subjects treated more than 6 years previously were still seropositive. The immune response is complex possibly resulting in immune modulation and suppression of antibody production during infection. However, once the cyst is removed specific antibody reactions can persist for several years (Zhang & McManus 2006). Alternatively subjects with a history of hydatid disease may be at greater risk of re-exposure.

Risk factors associated CE included poverty indicators, household size and duration of sickness. Associations with poverty indicators such as unemployment (Torgerson et *al.* 2003a) or low income (Budke *et al.* 2005a) have been reported previously. The numbers of days sick could result from subjects having either clinical hydatid disease or concomitant illness increasing susceptibility to infection. The former is more likely as

infection with *E. granulosus* would occur some time earlier, whereas the risk factors are contemporaneous. Furthermore, when ultrasound positive cases only are used as the dependent variable, only poor living standard is significant. These patients were unaware of their diagnosis and hence suggests that the number of days sick in a years is a result of recent treatment or clinical hydatid disease. Dog ownership or close contact with dogs was not a significant. Because most people reported close contact with dogs it is difficult to discriminate on this factor. The absence of clustering within households suggests that individual behaviour promotes transmission. The increased likelihood of large families having at least one case is a function of the increased probability of finding a case in a larger group.

The adjusted estimated annual incidence of 50 cases per 100,000 is high compared to officially reported incidence in southern Kazakhstan (Torgerson *et al.* 2002). This could indicate underreporting elsewhere or, more likely, that this community has a high incidence of CE as it was targeted because of the known high prevalence in dogs.

The combined adjusted prevalence and prevalence of individuals recently treated for echinococcosis at 1.3-1.9% is lower than on the Tibetan plateau where ultrasound prevalences approach 5% for both forms of the disease (Budke *et al.* 2004). Parasite data from purgation studies from dogs from Tibet is available (Budke, et al. 2005b), although there could be several other factors affecting the samples collected. Furthermore, the sensitivity of arecoline purgation is poor (Ziadinov *et al.* 2008). Nevertheless, there is a considerably higher abundance of *E. granulosus* and a similar abundance of *E. multilocularis*, compared to Tibetan dogs using the same method. However, there is considerably lower transmission of both parasites to humans. Only 9 Kazakh subjects reported that they allowed their dogs inside their homes. Alternatively the Tibetan population may be more susceptible due to greater poverty or concomitant disease.

Logistic regression for toxocarosis suggested males were more likely seropositive. The dogs in this community are mainly working dogs and hence have greater contact with males who work more with these animals. Disposing of dog faeces on the garden could result in contamination of home grown vegetables with *Toxocara* eggs. Otherwise no direct link with dog or cat ownership was found. The universality of dog contact in this community makes this factor difficult to discriminate. Seropositivity independently

decreased with age indicating a higher risk of infection in children consistent with other studies (Conde Garcia *et al.* 1989, Fillaux *et al.* 2007). This may be due to behaviour with children being at greater risk of environmental exposure to *Toxocara* eggs. There appears to be some clustering of infection at district level but it is not possible to hypothesise why. At a household level the association with better living standards is difficult to explain.

Toxoplasmosis infects approximately one third of humanity (Tenter *et al.* 2000). There is little data on the human prevalence in Kazakhstan, but livestock seroprevalences of up to 33% have been detected and between 1.6% and 5.6% of cats excrete oocysts (Beyer & Shevkunova 1986). In Uzbekistan seroprevalences in the human population of 14.6% and 24.6% have been reported (Asatova *et al.* 1993), with a risk of 0.4-0.6% seroconversion during pregnancy. This is a similar range to the present study. The lack of association with cat ownership suggests that infection may be acquired elsewhere such as uncooked meat or from the environment. A possible link to unsafe drinking water is consistent with other studies (Heukelbach *et al*. 2007, Bahia-Oliveira *et al.* 2003, Aramini *et al.* 1999, Bowie *et al.* 1997, de Moura et *al.* 2006). Household size indicates an increased probability of finding at least one seropositive individual and negative association with numbers of cattle may be an indicator of wealth. The association with household seropositivity for *Toxocara* may indicate a common risk factor for both zoonoses. One hypothesis could be that a significant proportion of human toxocariosis is due to *T. cati* and hence cats being the direct or indirect common factor.

**Acknowledgements**

The authors would like to thank (INTAS 03-51-5661 ), The Swiss National Funds (SCOPES Project Number 7KKPJ065622) and the NIH (TWO 1565-02) for the financial support of this study.

**References**

Aramini, J. J., Stephen, C., Dubey, J. P., *et al.* (1999). Potential contamination of drinking water with Toxoplasma gondii oocysts. *Epidemiology and Infection* 122, 305-315.

Asatova, M. M., Avdiukhina, T. I.& Gobunova, I. P. (1993). The results of a serological examination of the population for toxoplasmosis and the estimated incidence of congenital toxoplasmosis in 2 foci in Uzbekistan. *Med Parazitol (Mosk)*, 25-27

Bahia-Oliveira, L. M., Jones, J. L., Azevedo-Silva, J., *et al.* (2003). Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerging Infectious Diseases* 9, 55-62.

Beyer, T. V.& Shevkunova, E. A. (1986). A review of toxoplasmosis of animals in the U.S.S.R. *Veterinary Parasitology* 19, 225-243.

Bowie, W. R., King, A. S., Werker, D. H., e*t al.* (1997). Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. *Lancet* 350, 173-177.

Budke, C. M., Jiamin, Q., Zinsstag, J., *et al.* (2004). Use of disability adjusted life years in the estimation of the disease burden of echinococcosis for a high endemic region of the Tibetan plateau. *Am J Trop Med Hyg* 71, 56-64.

Budke, C. M., Jiamin, Q., Qian, W., et al. (2005a). Economic effects of echinococcosis in a disease-endemic region of the Tibetan Plateau. *Am J Trop Med Hyg* 73, 2-10.

Budke, C. M., Jiamin, Q., Craig, P. S., et al. (2005b). Modeling the transmission of *Echinococcus granulosus* and *Echinococcus multilocularis* in dogs for a high endemic region of the Tibetan plateau. *International Journal for Parasitology* 35, 163-170.

Budke, C. M., Deplazes, P.& Torgerson, P. R. (2006). Global socioeconomic impact of cystic echinococcosis. *Emerging Infectious Diseases* 12, 296-303.

Craig, P. S. (2006). Epidemiology of human alveolar echinococcosis in China. *Parasitology International* 55, S221-S225.

del Caprio, M., Moguilandsky, S., Cost, M., *et al.* (2000). Diagnosis of human hydatidosis. Predictive value of a rural ultrasonographic survey in an apparently healthy population. . *Medicina (Buenos Aires)* 60, 466-468.

Conde Garcia, L., Muro Alvarez, A.& Simon Martin, F. (1989). Epidemiological studies on toxocariasis and visceral larva migrans in a zone of Westen Spain. *Annals of Tropical Medicine & Parasitology* 83, 615-620.

Deplazes, P.& Gottstein, B. (1991). A monoclonal antibody against *Echinococcus multilocularis* Em2 antigen. *Parasitology* 103, 41-49.

Deplazes, P., Grimm, F., Sydler, T., *et al.* (2005). Experimental alveolar echinococcosis in pigs, lesion development and serological follow up. *Veterinary Parasitology* 130, 213-222.

Dendukuri, N.& Joseph, L. (2001). Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics* 57, 158-167.

Fillaux, J., Santillan, G., Magnaval, J. F., *et al.* (2007). Epidemiology of toxocariasis in a steppe environment: the patagonia study. *Amercan Journal of Tropical Medicine and Hygiene* 76, 1144-1147.

Fisher, M. (2003). Toxocara cati: an underestimated zoonotic agent. *Trends in Parasitology* 19, 167-170.

Gottstein, B.& Speiser, F. (1984). A collaborative study on larval excretory/secretorary antigens of Toxocara canis for the immunodiagnosis of human toxocariasis with ELISA. *Acta Tropica* 41, 361-372.

Gottstein, B., Jacquier, P., Bresson-Hadni, S., *et al.* (1993). Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *Journal of Clinical Microbiology* 31, 373-376

Hill, D. E., Chirukandoth, S.& Dubey, J. P. (2005). Biology and epidemiology of Toxoplasma gondii in man and animals. *Animal Health Reviews* 61, 41-61

Hoffmeister, B., Glaeser, S., Flick, H., et al. (2007). Cerebral toxocariasis after consumption of raw duck liver. *Amercan Journal of Tropical Medicine and Hygiene* 76, 600-602.

Heukelbach, J., Meyer-Cirkel, V., Moura, R. S., *et al.* (2007). Waterborne Toxoplasmosis, Northeastern Brazil. *Emerging Infectious Diseases* 13, 287-289.

Karanis, P., Kourenti, C.& Smith, H. (2007). Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health* 5, 1-38.

Mamuti, W., Sako, Y., Nakao, M., et al. (2005). Recent advances in characterization of Echinococcus antigen B. *Parasitology International* 55, s57-s62.

de Moura, L., Bahia-Oliveira, L. M., Wada, M. Y., et al. (2006). Waterborne toxoplasmosis, Brazil, from field to gene. *Emerging Infectious Diseases* 12, 326-329.

Nazirov, F. g., Ilkhamov, I. L.& Ambekov, N. C. (2002). Echinococcosis in Uzbekistan: types of problems and methods to improve treatment. *Medical Journal of Uzbekistan* 2/3, 2-5.

Oriol, R., Williams, J. F., Perez, E. M., *et al.* (1971). Purification of lipoprotein antigens of Echinococcus granulosus from sheep hydatid fluid. *American Journal of Tropical Medicine & Hygiene* 20, 569-574.

Ortona, E., Rigano, R., Margutti, P., et al. (2000). Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 22, 553-559.

Rapsch, C., Schweizer, G., Grimm, F., et al. (2006). Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. *International Journal for Parasitology* 36, 1153-1158

Shaikenov, B. S. (2006). Distribution and ecology of *Echinococcus multilocularis* in Central Asia. *Parasitology International* 55, S213-S219.

Stefanic, S., Shaikenov, B. S., Deplazes, P., *et al.* (2004). Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. *Parasitology Research* 92, 347-351.

Tenter, A. M., Heckeroth, A. R.& Weiss, L. M. (2000). *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology* 30, 1217-1258.

Torgerson, P. R., Shaikenov, B. S., Baitursinov, K. K., *et al.* (2002). The emerging epidemic of echinococcosis in Kazakhstan. *Transactions of the Royal Society of Tropical Medicine & Hygiene.* 96, 124-128.

Torgerson, P. R., Karaeva, R. R., Corkeri, N., *et al.* (2003a). Human cystic echinococcosis in Kyrgystan: an epidemiological study. *Acta Tropica* 85, 51-61.

Torgerson, P. R., Shaikenov, B. S., Rysmukhambetova, A. T., *et al.* (2003b). Modeling the transmission dynamics of *Echinococcus granulosus* in dogs in rural Kazakhstan. *Parasitology* 126, 417-424.

WHO Informal Working Group (2002). International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Tropica* 85, 253-261.

Zhang, W.& McManus, D. P. (2006). Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunology and Medical Microbiology* 47, 24-41.

Ziadinov, I., Mathis, A., Trachsel, D., *et al.* (2008). Canine echinococcosis in Kyrgyzstan: epidemiology and transmission analysis incorportaing diagnostic uncertainty. *International Journal for Parasitology* 38, 1179-1190.

.

.

.

Table 1. Observed and expected age and gender distribution of 3126 subjects. The expected distribution is based on extrapolation from general Kazakh population. $\chi^2$ = 320, $p<0.0001$

| Age of subjects | Male | | Female | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| <15 | 328 | 429 | 434 | 415 |
| 15-64 | 628 | 987 | 1455 | 1045 |
| >64 | 97 | 84 | 184 | 166 |
| Total | 1053 | 1500 | 2073 | 1626 |

Table 2. Results of ultrasound and serology for the diagnosis of cystic echinococcosis in 3126 tested subjects.

| | EgHF-ELISA –ve | EgHF-ELISA +ve | EgHF-ELISA + ve AgB-WB +ve |
|---|---|---|---|
| Ultrasound positive | 12 | 11 | 8 |
| History CE | 13 | 11 | 10 |
| No clinical CE | 3027 | 52 | 3 |

Table 3. Diagnostic parameters and estimated prevalence of CE. Although this assumed the ultrasound and EgHF-ELISA with AgB-WB confirmation both have specificities of 1, cross reactivity is possible with AE. However very little evidence of human AE was found in this population.

| | Sensitivity | Specificity | Positive predictive value | Prevalence |
|---|---|---|---|---|
| Ultrasound | 0.690 (0.420-0.892) | 1 | 1 | 0.0110 (0.007-0.0178) |
| EgHF-ELISA/AgB-WB | 0.331 (0.171-0.528) | 1 | 1 | |
| EgHF | 0.57 (0.33-0.78) | 0.990 (0.985-0.996) | 0.382 (0.202-0.719) | |
| Ultrasound/EgHF-ELISA/AgB-WB | 0.832(0.657-0.976) | | | |

Table 4 Prevalence of CE and diagnostic parameters of ultrasound and assuming specificity of EgHF-ELISA with AgB-WB confirmation is 1 and a prior specificity of ultrasound is 95.6% as described in the text.

| | Sensitivity | Specificity | Positive predictive value | Prevalence |
|---|---|---|---|---|
| Ultrasound | 0.673 (0.407-0.893) | 0.995 (0.992-0.998) | 0.380 (0.181-0.701) | 0.005 (0.002-0.011) |
| EgHF/AgB | 0.705 (0.319-0.984) | 1 | 1 | |
| EgHF | 0.943 (0.734-0.998) | 0.984 (0.979-0.990) | 0.243 (0.114-0.468) | |
| Ultrasound/EgHF/AgB | 0.930 (0.716-1) | | | |

Table 5. Significant factors associated with ultrasound positive or history of CE

| Variable | Regression coefficient (SE) | Odds ratio (95% CI) | P value |
|---|---|---|---|
| [1]Poor living standard | 0.716 (0.30) | 2.04(1.15-3.65) | 0.015 |
| [2]Number in the household | 0.143 (0.068) | 1.16(1.01-1.32) | 0.034 |
| [2]Days sick per year | 0.012 (0.003) | 1.012(1.0062-1.0179) | 0.000 |

1. Bivariate
2. Continuous variable

Table 6 Significant factors associated household zoonotic infection

| Zoonosis | Risk factor | Regression coefficient (SE) | Odds ratio (95% CI) | P value |
|---|---|---|---|---|
| CE | Number in household | 0.19 | 1.21 (1.04-1.41) | 0.04 |
| *Toxocara* infection | Good living standard | 0.438 | 1.55(1.08-2.22) | 0.02 |
| | *Toxoplasma*-seropositive in household | 0.579 | 1.76(1.24-2.48) | 0.002 |
| *Toxoplasma* infection | Water supply stream | 0.470 | 1.60 (1.02-2.51) | 0.04 |
| | Number of cattle | -0.05 | 0.94 (0.90-0.98) | 0.02 |
| | *Toxocara*-seropositivity in household | 0.538 | 1.71(1.21-2.43) | 0.003 |
| | Number in household | 0.13 | 1.14(1.05-1.23) | 0.003 |

Table 7. Significant factors associated with individual seropositivity to *Toxocara*

| Variable | Regression coefficient (SE) | Odds ratio (95% CI) | P value |
|---|---|---|---|
| [1]Gender Male | 0.293 (0.13) | 1.34(1.04-1.73) | 0.022 |
| [1]Throw away offal | 0.374(0.128) | 1.45(1.13-1.86) | 0.004 |
| [1]Smoker | -0.535(0.267) | 0.59(0.35-0.99) | 0.04 |
| [1]Dog faeces disposed on garden | 2.340 (0.727) | 10.38(2.50-43.16) | 0.001 |
| [2]Age | -0.020(0.003) | 0.98(0.97-0.99) | 0.000 |
| Random effects | Variance | | |
| District (random effect) | 0.147 | | |

1. Bivariate
2. Continuous variable

Table 8 Significant factors associated with individual seropositivity for *Toxoplasma gondii*

| | Regression coefficient (SE) | odds ratio (95% CI) | P value |
|---|---|---|---|
| Age | 0.014 (0.002) | 1.014(1.009-1.019) | 0.000 |
| Seropositive to CE | 0.749(0.272) | 2.115(1.239-3.610) | 0.006 |
| District (Random effect) | Var 0.263 | | |

Table 9. Results of purgation of 632 dogs from Jalanash sampled between 2003 and 2005

| | Number infected dogs (Prevalence) | Mean Abundance (95% bootstrap CIs) |
|---|---|---|
| *E. granulosus* | 85 (13) | 812 (244-1528) |
| *E. multilocularis* | 29 (5) | 72 (25-124) |
| *Taenia* spp. | 132(21) | 3.3 (1.4-5.7) |
| *Dipylidium caninum* | 29 (4) | 1.5 (0.7-2.6) |
| *Mesocestoides sp.* | 19 (4) | 0.6 (0.1-1.3) |
| *Toxocara canis* | 15 (2) | 0.06 (0.02-0.12) |
| *Toxascaris leonina* | 5 (1) | 0.01(0.001-0.02) |